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EXPERIMENTAL STUDIES ON EMBRYOGENESIS IN HYDROZOANS (TRACHYLINA AND SIPHONOPHORA) WITH DIRECT DEVELOPMENT

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ABSTRACT

The normal embryology of the trachymedusa *Aglantha digitale* and the siphonophores *Nanomia cara* and *Muggiaea atlantica* is described. Marking experiments on these embryos indicate that the site of first cleavage initiation corresponds to the oral pole of the oral-aboral axis. In *Muggiaea* the plane of the first cleavage corresponds to the plane of bilateral symmetry. Experiments in which presumptive aboral and oral regions are isolated from these embryos at different stages of development indicate that there is an early determination of different regions along this axis. Only the oral region of the *Muggiaea* embryo has the ability to regulate. These eggs have a pronounced centrolecithal organization. As a consequence of cleavage, the outer ectoplasmic layer of the egg ends up in the cells that form the ectoderm, while the inner or endoplasmic region of the egg ends up in the cells that form the endoderm. Experimentally created fragments of fertilized eggs that contain only ectoplasm differentiate to form an unorganized ectodermal cell mass, indicating that endoplasm is necessary in order to differentiate endoderm.

The process of embryogenesis in these animals and the developmental mechanisms they use are very different from those used by hydrozoans with indirect development. These embryos use a suite of developmental mechanisms which are very similar to those used by ctenophores. The significance of this similarity is discussed.

INTRODUCTION

From a developmental standpoint, the class Hydrozoa in the phylum Cnidaria appears to be quite diverse (Tardent, 1978). All of the experimental studies that have tried to define the mechanisms that underlie early embryogenesis in this group have been done on one order, the Hydroida. (Table I presents the taxonomic classification of the class Hydrozoa used in this paper.) On the basis of this experimental work a list of the mechanisms that mediate early embryogenesis in this group has emerged (see Discussion). One consequence of these mechanisms is that these embryos have a remarkable ability to regulate (Teissier, 1931, Freeman, 1981).

Most of the species in the order Hydroida have a complex polymorphic life cycle. In one major phase of the life cycle these animals are attached to a substrate and in the other major phase they are free swimming animals which function in a pelagic environment. The life cycle begins when an egg undergoes embryogenesis to generate a planula larva which undergoes metamorphosis to form a sessile polyp. Typically the polyp forms a colony that buds free swimming medusae. The medusae grow in size and develop gonads which form the gametes that are the basis for the next generation.

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TABLE I

Classification of the class Hydrozoa to a sub order level

order Hydroida	order Trachylina
Anthomedusae	Trachymedusae
Leptomedusae	Narcomedusae
Limnomedusae	Pteromedusae
order Milleporina	order Siphonophora
order Stylasterina	Cystonectae
	Physonectae
	Calycophorae
	order Actinulida

Not all orders of the class Hydrozoa have this kind of life cycle. In the order Trachylina the egg develops via a planula directly into a medusa; the polyp stage is absent or rudimentary. In the order Siphonophora the egg develops via a planula into a larva with both medusoid and polypoid characteristics that forms a colony composed of members of both types. The process of embryogenesis has been described for a number of species from these two direct developing orders (see Metschnikoff, 1874, 1886; Brooks, 1886 for the Trachylina; Metschnikoff, 1874, Russell, 1938, Carré, 1967, 1969 for the Siphonophora). Very little experimental work has been done on embryogenesis in the Trachylina and Siphonophora (however, see Zoja, 1895; Maas, 1908; Carré, 1969). These two orders are normally found in deep water in the open ocean; their life cycle occurs exclusively in a pelagic environment.

The present paper examines the process of embryogenesis in the trachymedusa *Aglantha digitale* and the siphonophores *Nanomia cara* (Physonectae) and *Muggiaea atlantica* (Calycophorae) from an experimental point of view. The results of these experiments indicate that these animals share only a few developmental mechanisms with the Hydroida. In each of these species there is a precocious segregation of developmental potential and the embryos have only a limited ability to regulate.

The Trachylina and Siphonophora are not the only cnidarian orders that develop directly. Some scyphozoan orders have a similar life cycle. Unfortunately no experimental work has been done on early embryogenesis in these animals. However, in the related radiate phylum Ctenophora, development is also direct. In these animals the product of embryogenesis is a larva, but in most species no major polymorphic change in the anatomy of the larva occurs as it grows into an adult. The embryology of these animals has been studied in some detail from an experimental viewpoint (see Reverberi, 1971 for a review). In this group there is also a precocious segregation of developmental potential. The life cycle of most ctenophores occurs exclusively in a pelagic environment. In the discussion section of this paper the developmental mechanisms used by the Hydroida and the Ctenophora will be compared. The significance of the demonstration that Trachylina and Siphonophora embryos have a suite of developmental mechanisms that are similar to those of the Ctenophora will then be discussed.

MATERIALS AND METHODS

Animals

Aglantha and *Nanomia* were collected from the Friday Harbor laboratory dock with a beaker attached to the end of a pole. These two species are not common in

the surface waters at Friday Harbor during the spring and summer seasons. Their distribution is patchy; during some years *Aglantha* appears to be much more abundant than during other years. *Aglantha* is always more common than *Nanomia*. *Muggiaea* eudoxids were collected by doing plankton tows 3–4 meters below the surface and half way up East Sound on Orcas Island. Eudoxids are present there in the last half of June, July, and August. Kozloff (1974) was used for identifying the species employed in this study. All of these species descriptions are based on animals found in the Atlantic Ocean. While these animals resemble those found in the Atlantic, there are some differences; it is not clear that these animals are identical to the Atlantic species.

Eggs were obtained through natural spawnings. It is difficult to predict the time of spawning for these species. Bowls containing the animals were checked at 30 minute to 2 hour intervals throughout the day for spawning. *Aglantha* tends to spawn between 0200–0400 and 1000–1200 hours. *Nanomia* tends to spawn one hour after it is brought into the light. In the siphonophores the testes tend to become opaque 2–3 hours prior to spawning. In *Aglantha* and in *Muggiaea* eudoxids the sexes are separate. In these species 2–4 sexually mature females were maintained in a bowl. When eggs were found they were collected and a culture was set up by adding sea water containing sperm from a bowl of males. A sexually mature *Nanomia* has both female and male gonads. In this work eggs were fertilized by the sperm from the same individual as they were spawned. The initiation of first cleavage was regarded as T_0 for the purpose of timing development.

The embryos were raised at 11–12°C. They were reared in millipore filtered pasteurized sea water in wells (0.5–1.5 ml vol.) of spot plates. In many of the experiments involving *Aglantha* blastomere isolates, 100 units/ml of penicillin was added to the sea water; this significantly improved viability. The siphonophore embryos frequently get caught at the air-water interface and are destroyed by surface tension forces. In some experiments polyethylene oxide was added to the sea water (0.1 g/10 ml) to increase its viscosity. The embryo develops normally in this medium, but moves very little; as a consequence it is much less likely to get caught at the air-water interface.

Experimental manipulations

Embryos were operated on in wells with a 2% agar bottom. Glass needles were used as knives to cut the embryos into parts.

Early cleavage stage embryos were marked with chalk particles (chalk was used because the vital dyes that were tried tended to diffuse throughout the early embryo). A suspension of small chalk particles was produced by placing a drop of sea water on a frosted glass slide and rubbing the tip of a stick of chalk in it. A small amount of this suspension was placed in one corner on the agar surface of an operating dish which was then filled with sea water. The part of the embryo surface to be marked was placed in contact with one or more chalk particles and gently pressed against the chalk with a blunt glass needle attached to a micromanipulator. This procedure firmly attaches the chalk to the embryo's surface. Embryos at later stages were marked with the stain nile blue; a 1% solution of the dye was prepared in distilled water. One or more points on the surface of the embryo were marked by using a micromanipulator to bring the open end of a fine capillary tube filled with 2% agar containing the dye in contact with the surface of the embryo for a few minutes. Novikoff (1938) gives directions for preparing these capillary tubes. The embryos tend to lose stain and there is a diffusion of the stain into the endoplasmic region; however, the dyed spot can usually be followed for 2–3 days. Since the embryos are translucent the dye spots

on the surface can be observed even when they are very light by viewing the embryo with a compound microscope under conditions of critical illumination. Frequently embryos were first marked with chalk and subsequently remarked by staining. Too much stain has a deleterious effect on the development of these embryos.

Eggs were centrifuged to create ectoplasmic and endoplasmic fragments. Centrifugation stratifies the contents of the egg. In these species the yolky endoplasm takes up a centripetal position and the ectoplasmic zone takes up a centrifugal position. Following stratification the eggs elongate and may split into endoplasmic and ectoplasmic fragments. If they do not separate into fragments they can be easily cut into fragments following centrifugation. *Aglantha* eggs were centrifuged in a mixture of 2 parts 1 molal sucrose and 1 part sea water for 15 minutes at 9500 rpm (10,800 $\times$ *g*). *Nanomia* eggs were centrifuged in a mixture of 1 part 1 molal sucrose and 1 part sea water for 10 minutes at 9500 rpm. The diameter of the fragments was measured with a screw micrometer eye piece.

Histological procedures

Embryos and larvae were fixed in 1% osmium in cold sea water for one hour, washed, dehydrated, and embedded in Epon. Sections were cut at 2 μm and stained with methylene blue and azure II (Richardson *et al.*, 1960).

Results

Normal development of Aglantha digitale

The normal development of *Aglantha* has not been described; however, Metschnikoff (1886) described the development of a related species, *Aglaura hemistoma* from the Mediterranean. Figure 1 presents a series of photographs which outline the development of *Aglantha*. The uncleaved egg has an average diameter of 139 μm (range 125–153 μm, sample size 24). There is a membrane around the egg which is closely applied to it; the embryo hatches out of the membrane during development (Fig. 1f). Polar bodies are not visible. Sections through fixed uncleaved eggs (Fig. 2a) show a central zone containing large endoplasmic granules and a peripheral region where these granules are absent. Cleavage is unipolar. The first two cleavages generate four equal blastomeres. A number of embryos were marked at either the site of origin of the first cleavage furrow (18 cases) or directly opposite this site (5 cases). These marking studies showed that the second cleavage is always initiated at the same site at the first cleavage. The third is unequal. Four micromeres are produced that are largely devoid of endoplasmic granules (Figs. 1b, 2b). The embryos with the chalk marks showed that the micromeres are given off opposite the site of first cleavage initiation. During the fourth cleavage (Fig. 1c) the four macromeres divide equatorially to form two tiers of macromeres along the axis specified by the first two cleavage furrows. It is hard to follow cleavage beyond this point.

Gastrulation takes place during the next 3–4 hours. During this period the cells that make up the micromere cap flatten and spread as a coherent layer over the macromeres to create an ectodermal cell layer which surrounds the yolky macromeres (Fig. 2c). The spreading movement can be followed by observing appropriately oriented embryos at 10–15 minute intervals using Nomarski optics. It is not clear that epibolic movement is the only mechanism of gastrulation; endoplasm free ectodermal cells may also be generated by a cytokinesis which occurs tangentially to the external cell membrane in some of the macromeres. As gastrulation takes place the embryo elongates

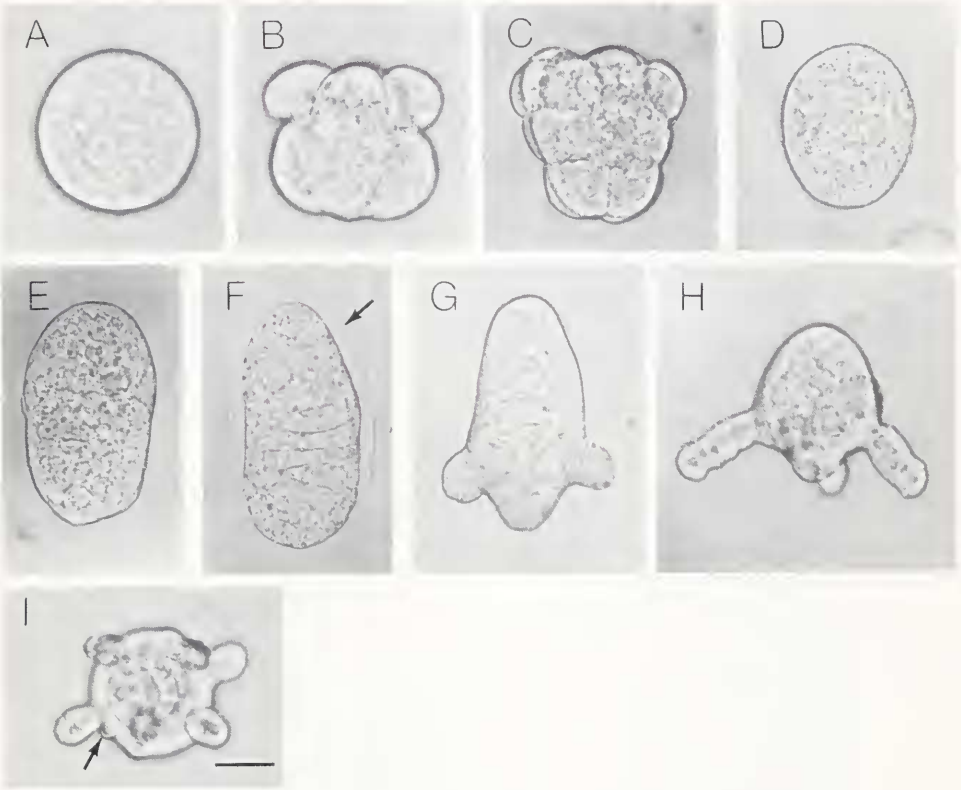

FIGURE 1. Normal development of *Aglantha*. A) Uncleaved egg. B) Eight cell stage. C) 16 cell stage D) Five hour embryo. E) Eight hour embryo. F) 24 hour embryo. The arrow indicates the membrane that surrounds the egg out of which the embryo is hatching. G) 40 hour embryo. Note the tentacle rudiments. H) 54 hour embryo. I) Oral view of three day old embryo. The tentacles are contracted. The arrow indicates a marginal sense organ. All photographs are at the same magnification. The bar indicates 50 μm.

(Fig. 1d). The marking experiments show that the direction in which the embryo elongates corresponds to the axis of the first two planes of cleavage.

After gastrulation is completed, the ectodermal cells begin to form cilia. Over the next 12 hour period the embryo hatches out of its membrane and begins to swim. The planula rotates around its long axis as it moves forward; it does not reverse its direction of movement. Experiments in which the chalk marks were replaced by dye marks at 8–10 hours of development (8 cases) show that the site where cleavage is initiated corresponds to the posterior or oral end of the planula.

Between 24 and 48 hours of development two tentacles begin to form opposite each other in the oral region of the planula (Fig. 1g). At this point the planula begins to transform into an actinula. Within a few hours after these two tentacles begin to form, two more tentacles start to appear opposite each other between the first pair of tentacles. During the next few days additional tentacles form. A tentacle is composed of both ectodermal and endodermal cells; the ectoderm contains both nematoblasts and nematocysts. The tentacles of an actinula are relatively rigid but they can contract and change positions. Ciliary tracts that run the length of the tentacle serve as the main locomotory organ of the actinula. The tentacles of the medusa have similar ciliary tracts which beat in a coordinated manner. After four days of development

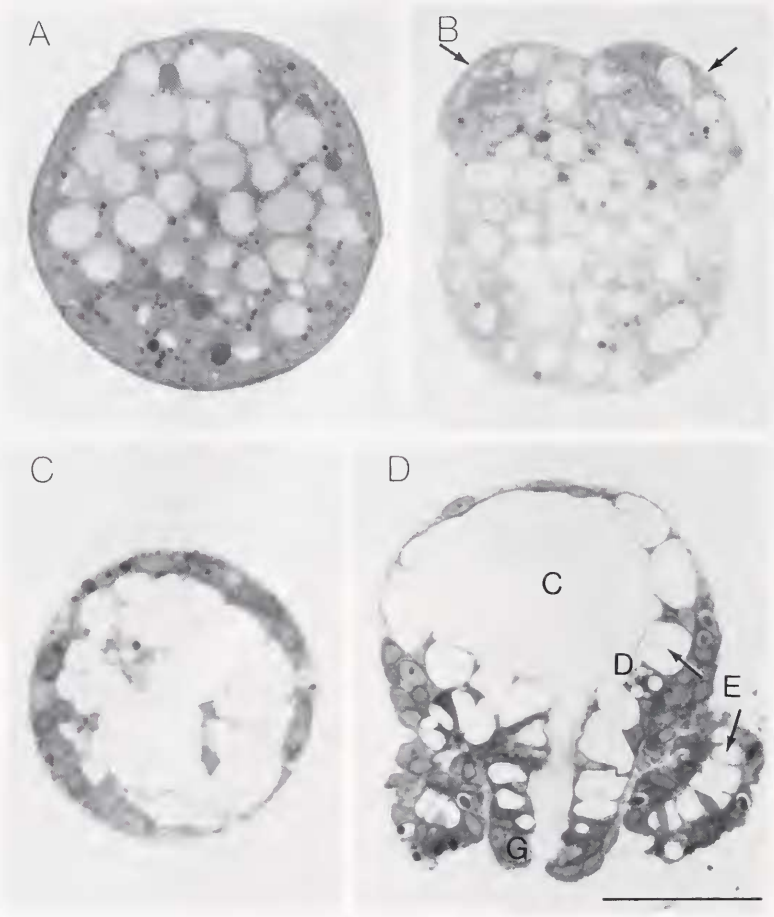

FIGURE 2. Normal embryology of *Aglantha*. A) Section through an egg. Note the endoplasmic granules. B) Section through an eight cell stage embryo. The arrows indicate the micromeres. Note the relative paucity of endoplasmic granules in the micromeres. C) Section through seven hour embryo. Note the ectoplasm containing ectodermal cells and the endoplasm containing endodermal cells. The ectodermal nuclei have nucleoli. Note the change in the morphology of the endoplasmic granules between the onset of cleavage and gastrulation. D) Section through a five day old larva. Note the gastrovascular cavity, C; the gland cells associated with the mouth G; the digestive gland cells, D; and the endodermal cells which line the gastrovascular cavity and make up the core of the tentacles, E. The bar indicates 50 μm.

marginal sense organs form between some of the tentacles. At the same time the tentacles start to form, the manubrium begins to differentiate at the oral end of the planula. At this site a heavily ciliated mouth forms between days two and three of development. Between 3 and 4 days of development manubrial gland cells form adjacent to the mouth. The endoderm, which is composed of highly vacuolated cells, organizes itself into an epithelium and a space which is continuous with the manubrium forms in the center of the larva. As these events occur a basement membrane forms between the ectodermal and endodermal cell layers. By five days of development gland cells with small vacuoles begin to appear between the large vacuolated endodermal cells in the oral region of the larva (Fig. 2d).

Experimental work on Aglantha

The ability of parts of embryos that normally form different germ layers or different regions of the actinula larva, to differentiate these structures when isolated was studied by doing the following experiments.

1) At the eight cell stage the micromeres and macromeres were isolated (Fig. 3a). Since the micromeres form ectoderm and the macromeres form both ectoderm and endoderm in the intact embryo, this experiment asks whether or not there is a segregation of germ layer specific developmental potential at the eight cell stage. Nineteen micromere and 25 macromere halves were raised for six days; four cases of each type were sectioned. All of the micromere isolates formed a compact sphere (Figs. 4a, 5a). The surface cells were ciliated but there was no indication of swimming polarity. In every case nematocysts were present. There was no indication of a basement membrane separating the external from the internal cells of the sphere. The development of the macromere isolates was more variable (Figs. 4b, 5b). The isolates were spherical to oblong in shape. All of them formed both ectoderm and endoderm; however, endodermal gland cells did not differentiate. In all of the cases the ectodermal cells appeared to be very thin in places or did not completely cover the endoderm. The ectodermal cells were ciliated; in 13 cases the isolates showed swimming polarity. Nematocysts were present in the ectoderm in 15 cases. Two of the isolates formed a stubby tentacle and one of these cases formed a manubrium.

2) At the 16 cell stage the embryo was cut into two parts in such a way that one isolate consisted of four oral macromeres and the other consisted of the micromeres and their adjacent macromeres (Fig. 3b). This experiment was done to find out if an embryo which lacks the endodermal cells that are normally present at the oral end of the embryo still has the ability to form tentacles and a manubrium. Twenty-two micromere and aboral macromere isolates were raised for six days; six of these cases were sectioned. Fifteen of these isolates formed normal actinula larvae (Fig. 4c); three cases formed a manubrium but no tentacles and one case formed tentacles but no manubrium. The three remaining isolates formed ectoderm containing nematocysts and endoderm, all of them showed swimming polarity. Sixteen oral macromere isolates were raised for six days; three of these cases were sectioned. These cases resembled the eight cell stage macromere isolates (Fig. 4d); however, they did not form nematocysts and no case showed swimming polarity.

3) At eight hours of development (Fig. 1e) (5 hours after the 16 cell stage), the embryo was cut into an oral and an aboral half (Fig. 3c). In order to identify each half, these cases were marked at the site of origin of the first cleavage furrow. At eight

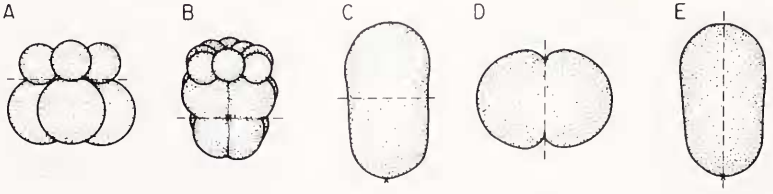

FIGURE 3. Operations performed to isolate parts of the *Aglantha* embryo at different stages of development. A) Eight cell stage; isolation of micromeres and macromeres. B) 16 cell stage; isolation of micromeres with aboral macromeres and oral macromeres. C) Eight hour embryo; isolation of oral and aboral halves. D) Two cell stage; isolation of individual blastomeres. E) Eight hour embryo; isolation of lateral halves. x, chalk mark placed at the site of origin of the first cleavage indicating the oral end of the embryo. The dashed line indicates how the embryo was cut.

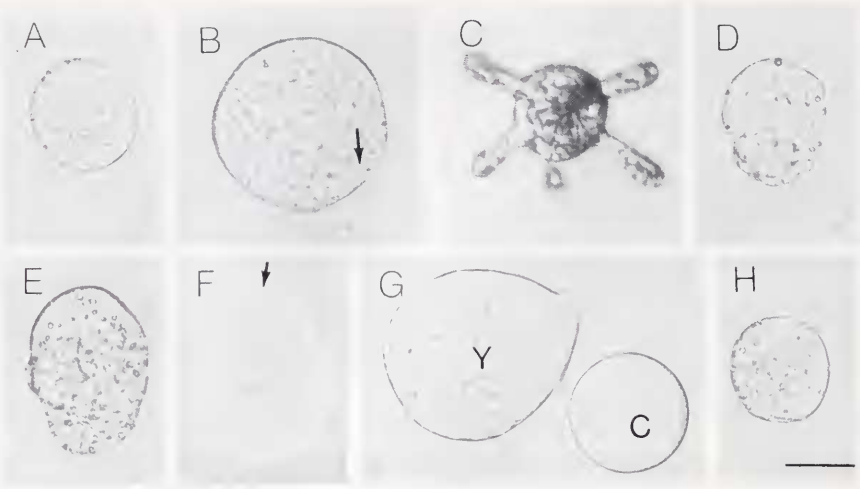

FIGURE 4. The development of isolates from *Aglantha* embryos. A) Five day old micromere isolate from eight cell stage embryo. Note the lack of endoderm. B) Five day old macromere isolate from eight cell stage embryo. The arrow indicates the boundary between the ectoderm and endoderm. C) Five day old micromere and middle macromere isolate from a 16 cell stage embryo. D) Five day old oral macromere isolate from 16 cell stage embryo. E) Five day old aboral half isolated from eight hour old embryo. F) Three day old isolate from two cell stage embryo. The arrow indicates the ectodermal cap. G) Y, yolky fragment and C, clear cytoplasmic fragment from a centrifuged egg. Note the nucleus in the clear cytoplasmic fragment. H) Four day old embryo from clear cytoplasmic fragment. Note the lack of endoderm. All photographs are at the same magnification. The bar indicates 50 μm.

hours of development both regions of the embryo have ectodermal and endodermal cell layers; a number of hours will elapse before there is an obvious indication of organogenesis. This experiment was done to find out if the aboral half of the embryo can regulate to form the tentacles and manubrium which are normally formed in the oral half of the embryo. Twenty-one aboral halves were raised for six days; eight of these cases were sectioned. All of the aboral halves developed swimming polarity. Eighteen cases showed no indication of tentacle or manubrium formation (Fig. 4e). Fourteen of these cases formed nematocysts at their oral end; the six embryos in this category that were sectioned had small vacuole endodermal gland cells. Three cases formed one or two stubby tentacles at their oral end, in all three cases nematocysts were present. None of these cases showed any indication of forming a manubrium, this point was checked by sectioning two of these cases. Sixteen oral halves were raised for six days. All of these cases formed a normal larva with tentacles and a manubrium.

4) Two kinds of control operations were performed. At the two cell stages each blastomere was isolated (Fig. 3d) and at the eight hour stage the embryo was cut into two lateral halves (Fig. 3e). These isolates contain both oral and aboral regions and ectodermal and endodermal germ layers or have the potential to form these germ layers. Twenty-eight two cell stage isolates were raised for six days; three of these cases were sectioned. All of these cases formed both ectodermal and endodermal germ layers. Eighteen of the isolates formed a ball of endodermal cells with a cap of ectoderm covering primarily one end of the endoderm (Figs. 4f, 5c). Nematocysts were frequently present in the ectodermal cell layer. Many of these isolates (14) showed swimming polarity. The ectodermal cap was always at the aboral end of the swimming isolate. (Eight isolates had a mark indicating where the first cleavage was

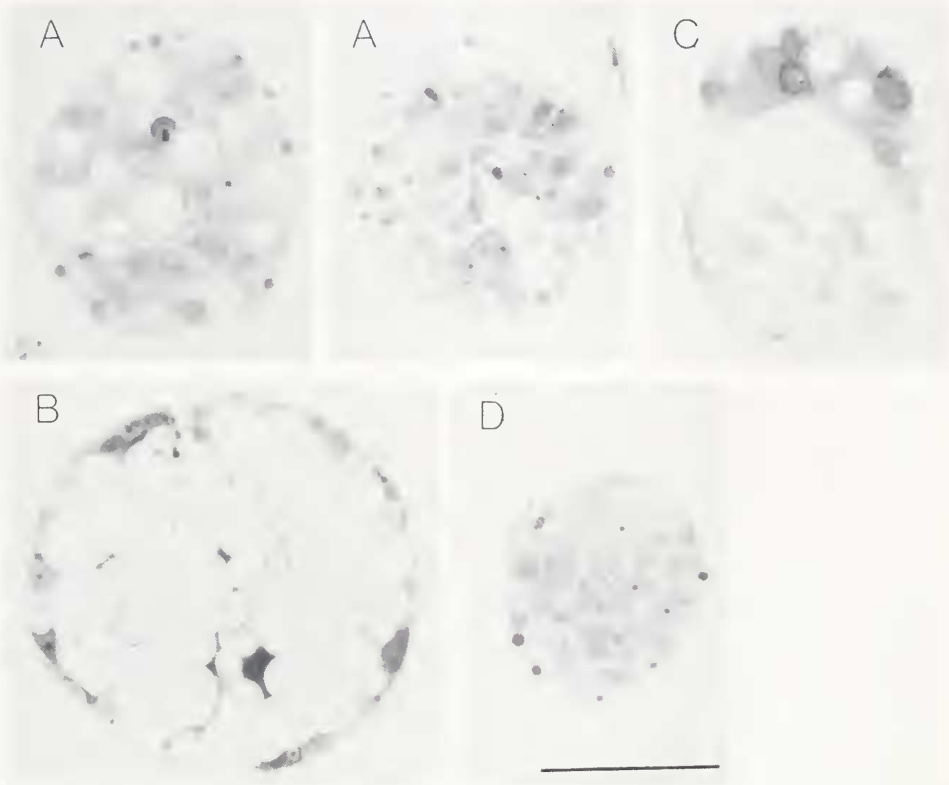

FIGURE 5. The histology of sectioned isolates from *Aglantha* embryos. A) Two five day micromere isolate from eight cell stage embryo. Note the lack of endoderm. B) Five day macromere isolate from eight cell stage embryo. Note the places where the ectoderm does not completely surround the endoderm. C) Five day isolate from two cell stage embryo. The endoderm is surrounded by ectoderm and there is an ectodermal cap at one end of the embryo. D) Four day embryo from clear cytoplasmic fragment. Note the lack of endoderm. All photographs are at the same magnification. The bar indicates 50 μm.

initiated; the cap was opposite the mark.) These cases probably failed to gastrulate properly. The remaining cases (10) formed more or less normal actinula larvae with one or more tentacles and a manubrium. Fifteen, eight hour lateral half isolates were raised for six days. Twelve of these cases formed actinula larvae with one or more tentacles and manubrium. These experiments show that the patterns of development seen in experiments 1–3 cannot be ascribed to the operative procedures used, but must reflect a program of differentiation inherent in the various regions of the embryo at the time these regions were isolated.

5) The last experiment investigated the effect of the yolky endoplasm on development. Nucleated egg fragments that lacked endoplasm were produced by centrifuging fertilized eggs and examining the ability of the resulting fragments to develop. Figure 4g shows the ectoplasmic and endoplasmic fragments produced by centrifugation. The average diameter of the ectoplasmic fragments was 90 μm (range 81–95 μm, sample size 35), while the average diameter of the endoplasmic fragments was 126 μm (range 109–156 μm, sample size 28). The ectoplasmic fragments contained approximately 27% of the egg volume. Five ectoplasmic fragments were sectioned; they

contained the nucleus and all of the visible egg constituents except the larger endoplasmic yolk granules. None of the endoplasmic fragments that were produced cleaved (115 cases). Sixty-two (38%) of the ectoplasmic fragments cleaved compared with approximately 70% for the untreated control eggs from the same batches. The first two cleavages of the ectoplasmic fragments are normal; however, at the third cleavage micromeres are not formed. There was no indication of gastrulation. After 24 hours of development a solid ciliated ball of small cells formed. Most of these isolates disintegrated between the second and third day of development. It was possible to raise 13 cases for five days (Figs. 4h, 5d); four of these cases were sectioned. They resembled eight cell stage micromere isolates, but they lacked nematocysts. They showed no swimming polarity; they lacked endoderm and showed no indication of manubrium or tentacle formation. This experiment shows that in the absence of endoplasm, endoderm will not differentiate.

Normal development of Nanomia cara *and* Muggiaea atlantica

The normal development of a Mediterranean species of *Nanomia* (Metschnikoff, 1874; Carré, 1969) and *M. atlantica* and a related Mediteranean species of *Muggiaea* (Metschnikoff, 1874; Russell, 1938) have been described. Figures 6 and 7 present a

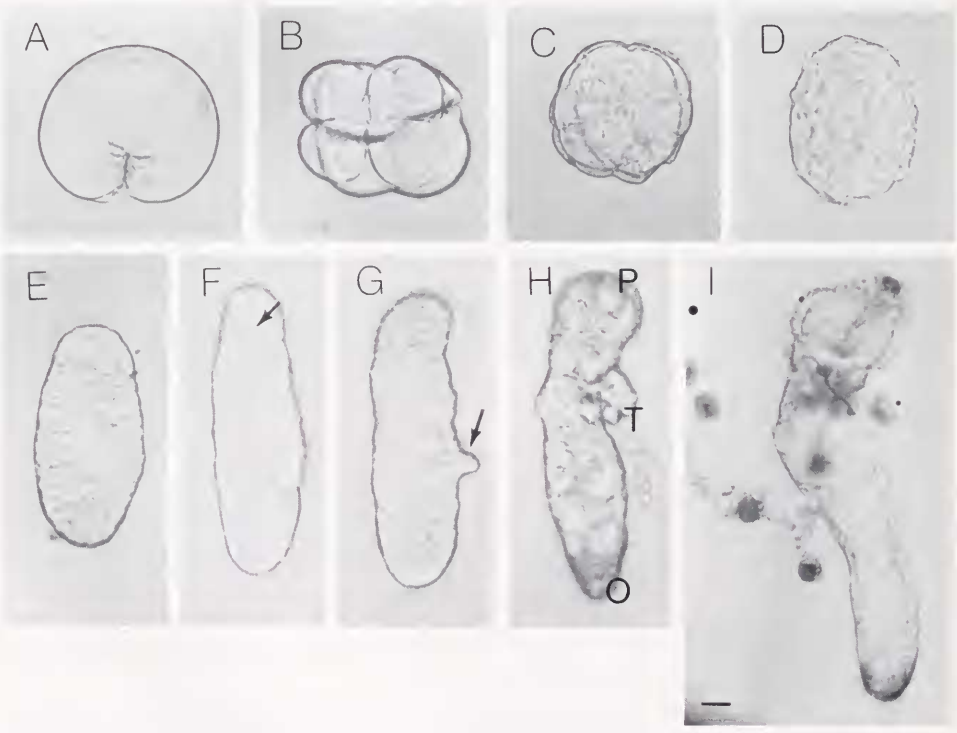

FIGURE 6. Normal development of *Nanomia*. A) First cleavage. Note the unipolar furrow. B) Eight cell stage. C) Six hour embryo. D) 12 hour embryo. E) 18 hour embryo. F) 44 hour embryo. Vacuolated cells have begun to form in the anterior region of the planula (arrow). Anterior and lateral endodermal thickenings are present. G) 57 hour embryo. The arrow indicates the tentacle rudiment. H) Four-and-a-half day old siphonula. Note the pneumatophore rudiment P, the tentacle with cnidobands at its base T, the oral pigment O, and the first indications of gastric cavity formation. I) Seven day old functional siphonula. Note the cnidobands on the tentacle. All photographs are at the same magnification. The bar indicates 50 μm.

series of photographs that outline the development of *Nanomia* and *Muggiaea*, respectively. The uncleaved *Nanomia* egg has an average diameter of 274 μm (range 252–292 μm, sample size 13), while the *Muggiaea* egg has an average diameter of 319 μm (range 307–331 μm, sample size 17). The *Muggiaea* egg is less dense than sea water. There are no membranes around these eggs. In *Muggiaea* the polar bodies are associated with an extracellular structure, the cupule (Carré and Sardet, 1981) that tends to fall off the egg shortly after fertilization. Sections through uncleaved eggs show a central zone containing large endoplasmic granules and a peripheral region where these granules are absent (Fig. 8a). The distinction between the ectoplasmic zone and the endoplasm is much sharper in these eggs than it is in the *Aglantha* egg. Cleavage is unipolar; the first two cleavages generate four equal blastomeres. In both species the site of origin of the second cleavage furrow was established by marking the site of origin of the first cleavage furrow or the point directly opposite this site. The second cleavage furrow occurred at the site of origin of the first cleavage furrow in 64% of the cases (sample size 22) for *Nanomia* and in 84% of the cases (sample size 18) for *Muggiaea*. In the remaining cases the second cleavage was initiated at the equator. The variable origin of the second cleavage furrow has been noted in *Nanomia* by Carré (1969), it has also been observed in other hydrozoans (Teissier, 1931; Freeman, 1981). The third cleavage is always perpendicular to the preceding cleavage and gives rise to two tiers of blastomeres with four equal sized cells in each tier (Figs. 6b, 7b).

Gastrulation begins following the 64 cell stage. Prior to gastrulation each blastomere contains part of the initial surface of the egg. The cell nucleus and the ectoplasmic region of the egg are found here. Gastrulation occurs when a tangential cell division gives an external daughter cell that contains the ectoplasmic region and an internal daughter cell that contains the endoplasm (Fig. 8b–d). Gastrulation is completed by six to seven hours of development in both species.

The *Nanomia* embryo begins to elongate between 12 and 18 hours of development; cilia develop and the embryo begins to show swimming polarity. The embryo has now transformed into a planula. Experiments in which the site of origin of the first cleavage furrow was marked show that this region corresponds to the posterior end of the planula (16 cases). During the next six hour period (18–24 hours of development) the embryo continues to elongate and an endodermal thickening begins to develop at the anterior end and along one side of the planula. At the same time the anterior ectodermal cells begin to enlarge (Fig. 6f). Between 42 and 54 hours of development the ectoderm at the anterior end of the planula invaginates and begins to form the pneumatophore, while a tentacle begins to grow out from the side of the planula where the endoderm has thickened. At this point the planula begins to transform into a siphonula larva. By seven days a feeding siphonula has developed. During this period the pneumatophore begins to secrete gas. Red pigment cells form at the posterior end of most larvae and a mouth with associated muscle and gland cells forms at this site. The large endodermal cells that filled the interior of the larva disappear and a gastric cavity forms in their place. Cnidobands form at the base of the tentacle and take up positions on the tentacle.

The *Muggiaea* embryo begins to form cilia between six and eight hours of development. By 12–15 hours the embryo shows swimming polarity and an endodermal thickening forms along one side of the planula; during this period the embryo also begins to elongate. At about 24 hours of development an invagination begins to form in the anterior part of the lateral endodermal thickening, this is the first indication of nectophore development; in the lateral band just below the invagination a bulge forms, this is the first indication of tentacle formation. Experiments in which the site

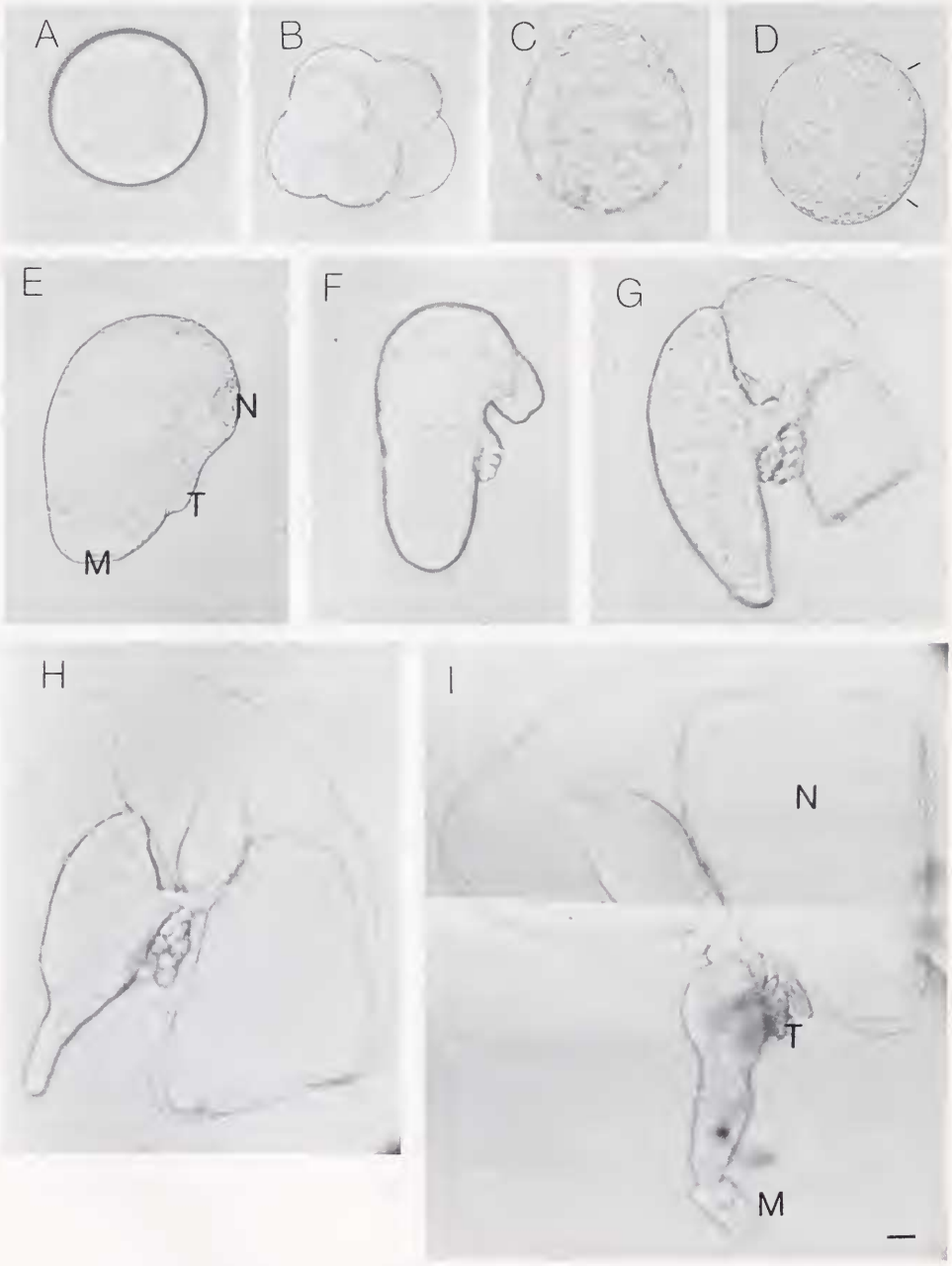

FIGURE 7. Normal development of *Muggiaea*. A) Uncleaved egg. B) Eight cell stage. C) Six hour embryo. D) 15 hour embryo. The brackets indicate the lateral endodermal thickening. E) 28 hour embryo. The nectophore and tentacle rudiments are beginning to form. F) 43 hour embryo. G) Two-and-a-half day old larvae. The nectophore is functional. H) Three-and-a-half day old larvae. The mouth is forming. I) Four-and-a-half day old larvae. Cnidobands are present on the tentacle. N, nectophore or rudiment; T, tentacle or rudiment; M, mouth or rudiment. All photographs are the same magnification. The bar indicates 50 μm.

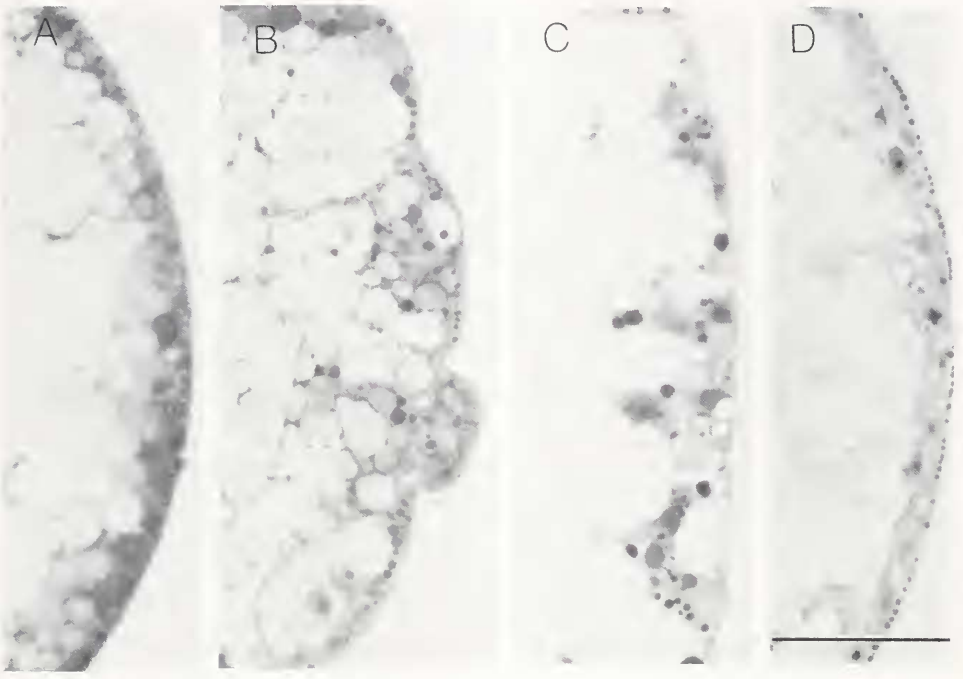

FIGURE 8. Normal embryology of *Nanomia* and *Muggiaea*. A) Section through part of a *Nanomia* egg. Note the large endoplasmic granules and the sharp transition between the ectoplasm and the endoplasm. B) Section through the peripheral region of a six hour *Nanomia* embryo. C) Section through the peripheral region of an 18 hour *Nanomia* embryo. D) Section through the peripheral region of an 18 hour *Muggiaea* embryo. C) and D) are similar. Note the ectoplasm containing ectoderm cells. *Muggiaea* has cortical granules under the outer ectodermal cell membrane; these were initially just under the egg membrane. The nuclei of these cells have nucleoli. The primary endoderm is made up of large endoplasm containing cells. The bar indicates 50 μm.

of origin of the first cleavage furrow was marked show that this region corresponds to the posterior end of these larvae (17 cases).

In both *Muggiaea* and *Nanomia* the formation of the lateral endodermal thickening and organogenesis in this region give the larva a bilateral character. In *Muggiaea* the relationship between the plane of the first cleavage and the plane of bilateral symmetry was studied by placing a series of chalk marks around the egg on the first cleavage furrow and examining the positions of the chalk marks in the 24 hour larva. Over 30 cases were marked, but only six cases were suitable for analysis because in most cases the chalk tends to get displaced from the surface of the planula when it begins to swim. In each of these six cases one side of the circle of chalk marks was coincident with the lateral endodermal thickening. An attempt to do this experiment in *Nanomia* failed because a much longer period elapses before an unambiguous lateral thickening develops and too many of the chalk granules were lost.

Between days one and four of *Muggiaea* development the nectophore rudiment grows rapidly and transforms into a functional locomotory organ. During this same period the somatocyst forms, the tentacle rudiment transforms into a functional tentacle and cnidobands form and take up positions on the tentacle. A mouth forms at the posterior end of the larva and the large endodermal cells that filled the interior of the planula disappear and a gastric cavity forms in their place.

Experimental work on Nanomia *and* Muggiaea

1) The first set of experiments on the siphonophore embryos were done to determine when the various regions along the oral-aboral axis, which will become the mouth, tentacle, and pneumatophore or nectophore, are specified to form these structures. The embryos were cut into oral and aboral halves at various times from the eight cell stage on and these halves were raised to see how they differentiated. Figure 9 indicates how these operations were done. When these operations were done at early stages of development (prior to 20 hours), the site of origin of the first cleavage furrow was marked so that the oral end of the embryo could be unambiguously identified. After an operation at the eight cell stage, gastrulation appeared to take place in both pieces at the normal time. When the operation was done after gastrulation, the ectodermal covering spread over the yolky endoderm cells within an hour. During this period the isolate sometimes lost one or two large endodermal cells. If cell loss was excessive the case was discarded. The results of these experiments are summarized in Tables II and III.

The results indicate that in *Nanomia* the specification of the mouth, tentacle, and pneumatophore forming regions along the oral-aboral axis has already occurred by the eight-cell stage of development. Aboral halves produced at this stage and later stages correspond to the anterior third of the siphonula (Fig. 10a). Most of the surface of these isolates is covered with large vacuolated cells. There is frequently a pneumatophore at the anterior end of these isolates and a rudimentary tentacle at the posterior end; frequently pigment cells are found at the posterior end but a mouth does not form. This point was checked by sectioning three of these cases. The muscle and gland cells that are characteristic of the mouth were not present. Oral isolates produced at the eight cell stage and later corresponded to the posterior two thirds of the siphonula (Fig. 10b). In most cases they have a tentacle at their anterior end (there may also be a few large vacuolated cells in this region) and a mouth and

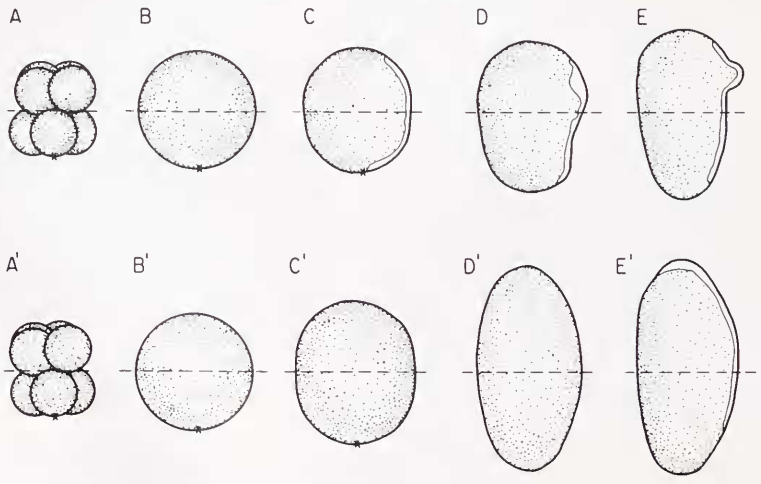

FIGURE 9. Operations performed to isolate oral and aboral halves of *Muggiaea* and *Nanomia* embryos at different stages of development. A–E, *Muggiaea*. A'–E', *Nanomia*. A) Eight cell stage. B) Six to seven hour embryo. C) 15–17 hour embryo. D) 22–24 hour embryo. E) 30–36 hour embryo. A') Eight cell stage. B') Six to seven hour embryo. C') 12–13 hour embryo. D') 18–19 hour embryo. E') 31–41 hour embryo. x, chalk mark placed at the site of origin of the first cleavage indicating the oral end of the embryo. The dashed line indicates how the embryo was cut.

TABLE II

The differentiation of Muggiaea *embryo halves isolated at different times during development*

Isolate type	Time of isolation	Number of cases	Corresponding member of pair	Kind of differentiation		
				Nectophore[1]	Tentacle[2]	Mouth
Oral	8-cell st.	4	2	4 (2)	4 (2)	4
	6–7 h	6	4	6 (3)	5 (3)	6
	15–17 h	4	2	4 (2)	4 (3)	4
	22–24 h	4	4	1 (0)	4 (3)	4
	30–36 h	3	2	0	3 (3)	3
Aboral	8-cell st.	2		2 (2)	0	0
	6–7 h	4		4 (3)	0	0
	15–17 h	3		3 (3)	0	0
	22–24 h	5		5 (5)	0	0
	30–36 h	4		4 (4)	0	0
Lateral	2-cell st.	22	9	22 (22)	21 (20)	22
	8-cell st.	1	0	1 (1)	1 (1)	1
	6–7 h	4	1	4 (3)	3 (2)	4
	22–24 h	2	1	2 (1)	2 (1)	2
	30–36 h	5	2	5 (4)	5 (4)	5
Dorso-ventral	6–7 h	10	4	10 (8)	10 (8)	10
Dorsal	15–17 h	5	3	4 (2)	4 (1)	5
	22–24 h	5	3	3 (1)	4 (0)	5
	30–36 h	1	1	0	1 (0)	1
Ventral	15–17 h	7		7 (6)	7 (6)	7
	22–24 h	7		7 (6)	6 (4)	7
	30–36 h	1		1 (1)	1 (1)	1

[1] The parenthesis indicates the number of cases that formed functional nectophores.
[2] The parenthesis indicates the number of cases that formed tentacles with nematoband brackets.

pigment cells at their posterior end. The *Nanomia* data can also be analyzed by examining the 24 examples of pairs of aboral and oral isolates from the same embryo. The tentacle forming region is found in the zone between the oral and aboral halves. Usually the tentacle is better developed in the oral isolate than it is in the aboral isolate. In those cases (3) where the tentacle is well developed in the aboral isolate it is rudimentary in the oral isolate. There is no indication that both halves develop more complete tentacles when they are isolated at an early stage *versus* a later stage. However, there is a tendency for oral isolates to differentiate large vacuolated cells more frequently when they are isolated at early stages rather than later stages. This suggests that the region which will differentiate large vacuolated cells may not have been definitively positioned along the oral-aboral axis of the embryo by the eight cell stage. The only feature which regulates its position along the oral-aboral axis is the pigment cells. These regularly form at the most posterior end of aboral halves regardless of the time at which these halves were isolated.

The results of the isolation experiments involving oral and aboral halves of the *Muggiaea* embryo are more complex. Aboral halves produced at the eight cell stage and later differentiated only the nectophore (Fig. 10c); these nectophores attain the size of nectophores from an intact embryo. This result suggests that the aboral half

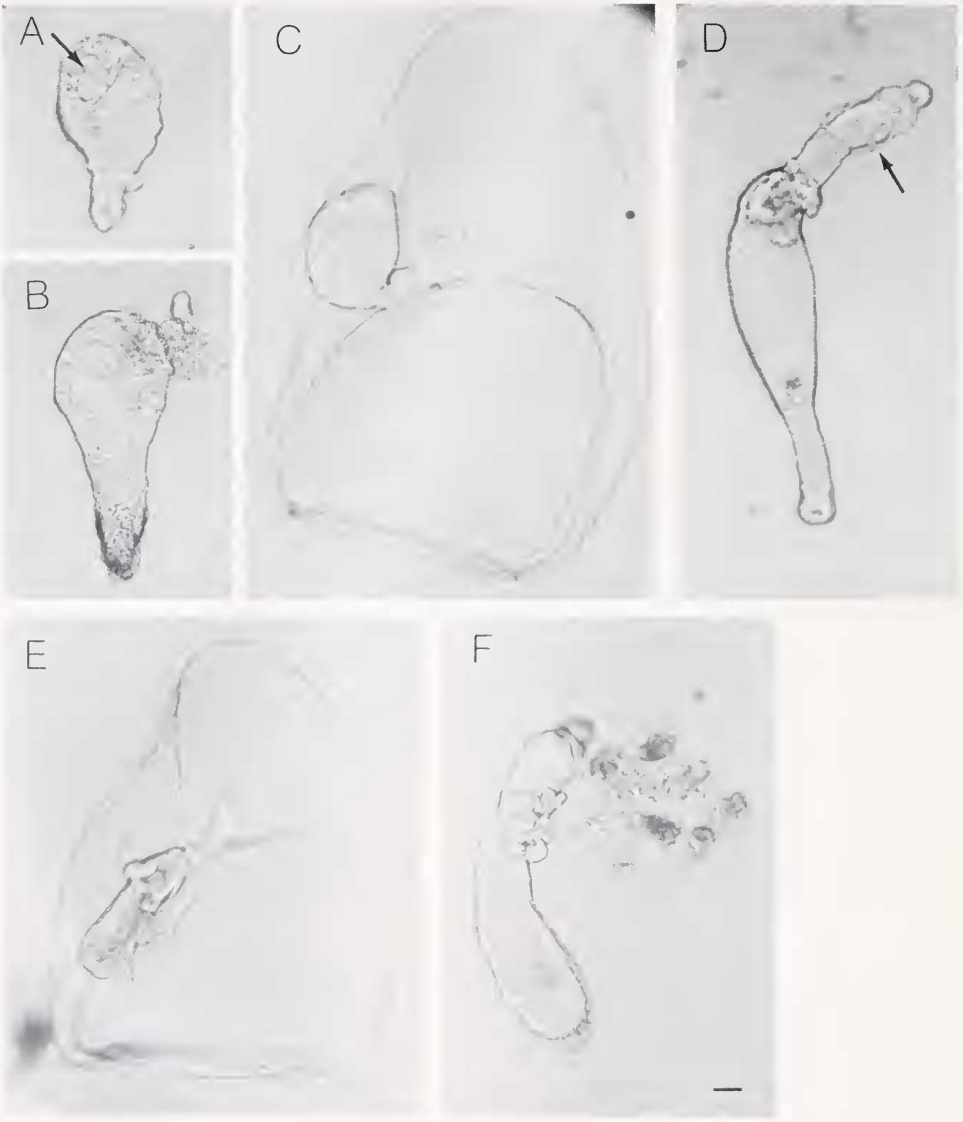

FIGURE 10. The development of aboral and oral isolates from *Nanomia* (A–B) and *Muggiaea* (C–F) embryos. All isolates are seven days old. A) Aboral half from eight cell stage embryo. Note the anterior pneumatophore rudiment (arrow). B) Oral half from same eight cell stage embryo as (A). Note the anterior tentacle with a cnidoband and the posterior mouth and pigment cells. C) Aboral half from eight cell stage embryo. Note the lack of a mouth and tentacle. D) Oral half from 15 hour embryo. The embryo has a mouth and a tentacle rudiment. The arrow points to an abnormal nectophore rudiment. E) Oral half from the same eight cell stage embryo as (C). Note the mouth and tentacle. F) Oral half from 22 hour embryo. The embryo has an anterior protrusion, a tentacle with a position along the body which is more anterior than normal and a posterior mouth. All photographs are at the same magnification. The bar indicates 50 μm.

of the embryo is specified to form the nectophore some time prior to the eight cell stage. Oral isolates produced at early developmental stages can form a normal larva with a nectophore, tentacle, and mouth. While the larva is smaller than normal, the

parts show the correct proportions (Fig. 10e). However, in half of these cases the nectophore was smaller than normal and nonfunctional or rudimentary (Fig. 10d). Since many of these oral isolates with a nectophore have a corresponding aboral isolate from the same embryo that has also formed a nectophore, the oral isolate must have formed a nectophore as a consequence of a regulatory adjustment that occurred as a result of the operation. Between 15 and 22 hours of development there is a marked decrease in the ability of the oral halves to differentiate a nectophore. These cases form a tentacle at their anterior end and a posterior mouth (Fig. 10f).

These experiments show that in both the *Muggiaea* and *Nanomia* embryos there is an early specification of the ways in which the different regions will differentiate along the oral-aboral axis of the future larva. However, in *Muggiaea* differentiation of part of the oral region of the embryo can be respecified until just before the first indications of organogenesis.

2) Since the different regions along the oral-aboral axis of the *Nanomia* embryo appear to be specified some time before the eight cell stage, an attempt was made to bracket the time period when specification occurs. Eggs which were undergoing their first cleavage (14 cases) and two cell stage embryos that were marked at the site of first cleavage initiation (5 cases) were cut into oral and aboral halves (Fig. 11). In each case only the oral half contained the nuclei, and thus it was the only half that developed. When an operation was done the diameter of each fragment was measured to calculate the relative volume of the oral isolate.

The results of these experiments are presented in Table III. Unlike the oral isolates produced at the eight-cell stage and at later stages, a substantial proportion of these cases formed large vacuolated cells and a pneumatophore. These cases can be further categorized by examining the kinds of structures that differentiate and the proportions of the larvae. Six cases formed miniature larvae of normal proportions (Fig. 12a). Ten cases formed the components of a normal larva but the anterior region was abnormally small (Fig. 12b). About half of these cases looked like the eight cell stage oral isolate that had a few large vacuolated cells at its anterior end. The anterior end of the other isolates was better developed. There were also three cases that did not form large vacuolated cells or a pneumatophore (Fig. 12c). There did not appear to be a correlation between the time of the operation or where the cut was placed with reference to the cleavage furrow and the kind of larva that differentiated. However, larger isolates tended to form more normal larvae than smaller oral isolates (Fig. 13). This experiment suggests that the specification of different regions along the oral-aboral axis of the *Nanomia* embryo is either taking place during the first cleavage and the two cell stage, or that it has occurred prior to this time, but the oral region

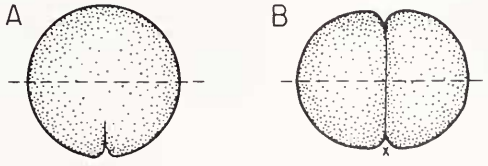

FIGURE 11. Operations performed to isolate oral portions of first cleavage and two cell stage *Nanomia* embryos. A) First cleavage. B) Two cell stage. In both operations the plane of the cut was perpendicular to the oral-aboral axis of the embryo; however, the position of the plane along the oral-aboral axis varied from case to case giving oral isolates of varying size. When operations were done on embryos that were undergoing their first cleavage in some cases the cut was made through the first cleavage furrow, in other cases the cut was made before the furrow reached that point. x, chalk mark placed at the site of the origin of the first cleavage indicating the oral end of the embryo. The dashed line indicates how the embryo was cut.

TABLE III

The differentiation of Nanomia *embryo halves isolated at different times during development*

Isolate type	Time of isolation	Number of cases	Corresponding member of pair	Pneumatophore	Kind of differentiation			
					Vacuolated cells	Tentacle[1]	Mouth	Posterior pigment
Oral	8-cell st.	9	8	0	3	4 (1)	9	7
	6–7 h	5	5	0	1	4 (1)	4	2
	12–13 h	7	7	0	1	7 (2)	7	6
	18–19 h	7	2	0	1	7 (2)	7	5
	31–41 h	2	2	0	0	2 (2)	2	2
Aboral	8-cell st.	9		7	9	4 (0)	0	4
	6–7 h	7		3	7	3 (0)	0	0
	12–13 h	7		6	7	5 (0)	0	1
	18–19 h	3		1	3	3 (0)	0	3
	31–41 h	6		5	6	6 (1)	0	5
Lateral	2-cell st.	16	4	7	16	16 (4)	16	12
	8-cell st.	8	4	5	7	7 (0)	7	4
	6–7 h	6	3	0	5	4 (0)	5	2
	12–13 h	6	3	4	6	6 (2)	6	4
Oral	1st cleavage-2 cell	19		9	16	18 (4)	19	17

[1] The parenthesis indicates the number of cases that formed tentacles with nematoband brackets.

of the *Nanomia* embryo can regulate in much the same way that the oral region of the *Muggiaea* embryo regulates at later developmental stages.

3) As a control experiment embryos were cut into lateral halves at the two cell

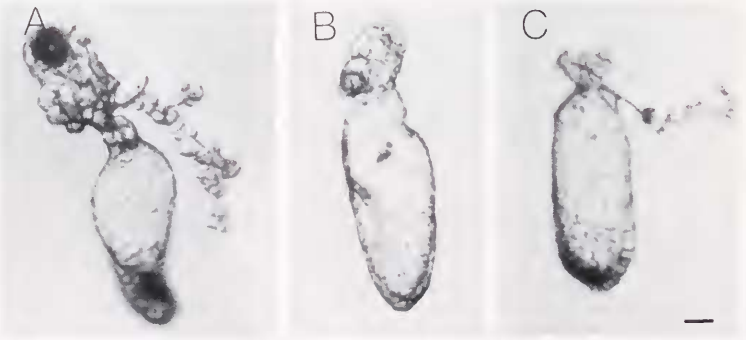

FIGURE 12. The development of oral isolates from *Nanomia* eggs undergoing their first cleavage. All isolates are seven days old. A) Normal larvae. The pneumatophore has secreted a gas bubble. This case developed from an egg fragment with 53% of the volume of a normal egg. B) Larvae with a reduced aboral end; a pneumatophore rudiment is present. This case developed from an egg fragment with 49% of the volume of a normal egg. C) Larva which lacks an aboral end. This case developed from an egg fragment with 50% of the volume of a normal egg. All of these cases developed from eggs that were cut through a non-furrow region when the furrow was a third of the way across the egg. All photographs are at the same magnification. The bar indicates 50 μm.

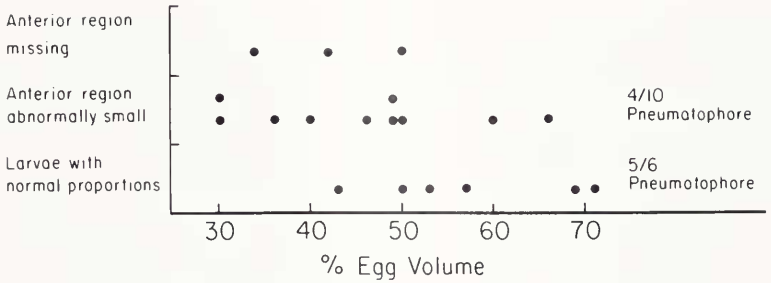

FIGURE 13. Graph relating the development of oral isolates from first cleavage and two cell stage *Nanomia* embryos to the size of the isolate.

stage and later developmental stages (Fig. 14). All of the operations on *Nanomia* embryos were performed before the development of the lateral thickening. Since the point where the lateral thickening will develop in these embryos is not known, it is more accurate to say that these embryos were cut along their oral-aboral axis. The *Muggiaea* embryos were cut into lateral halves. Since the first cleavage furrow defines the plane of bilateral symmetry the blastomere isolation experiments at the two cell stage produces lateral halves. The experiments at the eight cell stage and at six to seven hours of development were performed on embryos in which the first cleavage furrow was marked. At later stages the lateral thickening was obvious. Virtually all of these cases developed into normal larvae regardless of the stage when the operation was performed (Table II). Each pair of lateral halves from the same embryo always form the same structures. This experiment shows that the results obtained when these embryos are cut into oral and aboral halves cannot be ascribed to the operation *per se*.

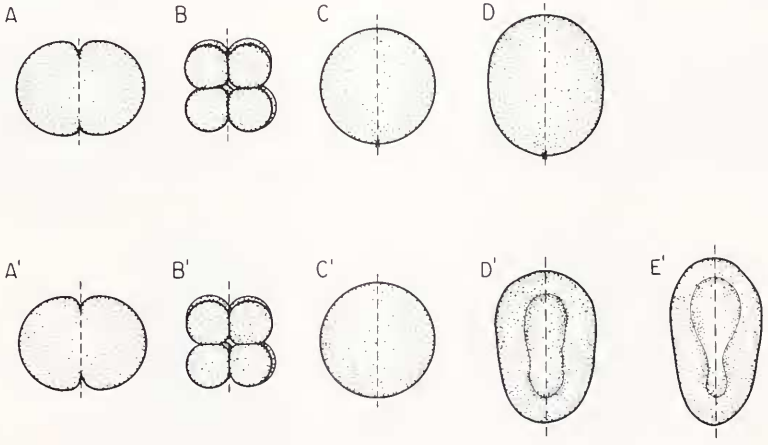

FIGURE 14. Operations performed to isolate lateral halves of *Nanomia* and *Muggiaea* embryos at different stages of development. A–D, *Nanomia*. A′–E′, *Muggiaea*. A) Two cell stage. B) Eight cell stage. C) Six to seven hour embryo. D) 12–13 hour embryo. A′) Two cell stage. B′) Eight cell stage. C′) Six to seven hour embryo. D′) 22–24 hour embryo. E′) 30–36 hour embryo. x, chalk mark placed at the site of origin of the first cleavage indicating the oral end of the embryo. The dashed line indicates how the embryo was cut. Embryos B′ and C′ were cut along a set of chalk marks that indicate the plane of the first cleavage. The ventral thickening was used to orient embryos D′ and E′.

4) The region along the oral-aboral axis where the lateral thickening forms is referred to as the ventral side of the embryo. The differentiation of the dorsal and ventral sides of the *Muggiaea* embryo was studied by cutting these embryos into halves along their frontal plane at various time periods during development (Fig. 15). The earliest stage when this operation was done was at six to seven hours of development on embryos in which the first cleavage furrow was marked. At this stage it is not possible to distinguish between a dorsal and a ventral side. All of these cases developed into normal larvae (Table II). In two pairs of isolates from the same embryo the chalk marks stayed on until the ventral thickening had formed. In both cases both members of each pair formed their ventral thickening under the same chalk mark, indicating that the outer surface of either side of the embryo is capable of becoming the ventral side. The other operations were performed after the ventral thickening had formed (15–36 h) (Table II). At all time periods when the operation was done the ventral halves formed normal larvae (Fig. 16a). The behavior of the dorsal halves depended upon when the operation was performed. When dorsal halves were produced at 15–17 hours of development a ventral thickening quickly formed opposite the cut and in most cases a nectophore and tentacle formed. When dorsal halves were produced at 22–24 and at 30–36 hours of development the ventral thickening took much longer to form and there was a marked decline in the ability of these halves to form a nectophore even though they formed a rudimentary tentacle (Fig. 16b). These experiments show that both of the regions defined by the plane of bilateral symmetry as potential dorsal or ventral sides of the embryo have the capacity to become the ventral side of the embryo. Even after the ventral side of the embryo has begun to differentiate, the dorsal side which is morphogenetically quiescent can differentiate as a ventral side. One of the embryos that was to be used for these operations at 22 hours of development illustrates this point in a different way. The glass needle that was used broke during the operation and only the aboral end of this embryo was cut along the frontal plane. The cut was rather jagged, however it healed over. Subsequently an endodermal thickening developed on the dorsal side of the embryo opposite the region where the nectophore would form on the ventral side. The embryo went on to form two nectophores (Fig. 16c).

Experiments have not been done that address the issue of dorsal ventral specification in *Nanomia*, however a few of the embryos operated on in experiment 3 must have been cut along or close to the presumptive frontal plane. Since both halves developed normally in all cases one can tentatively conclude that at the time the operations were performed the presumptive dorsal side (if it exists) can still regulate.

5) The last experiment investigated the effect of the yolky endoplasm of the

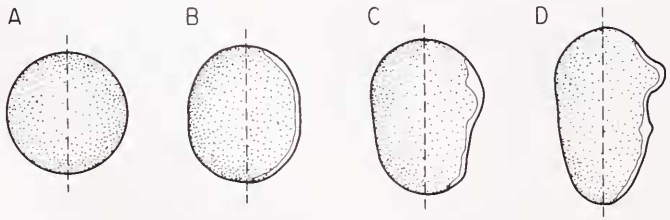

FIGURE 15. Operations performed to isolate dorsal and ventral halves of *Muggiaea* embryos at different stages of development. A) Six to seven hour embryo. This embryo was cut along the oral-aboral axis in a plane perpendicular to a set of chalk marks that indicate the plane of the first cleavage. B) 15–17 hours of development. C) 22–24 hours of development. D) 30–36 hours of development. The ventral thickening was used to orient embryos B–D. The dashed line indicates how the embryo was cut.

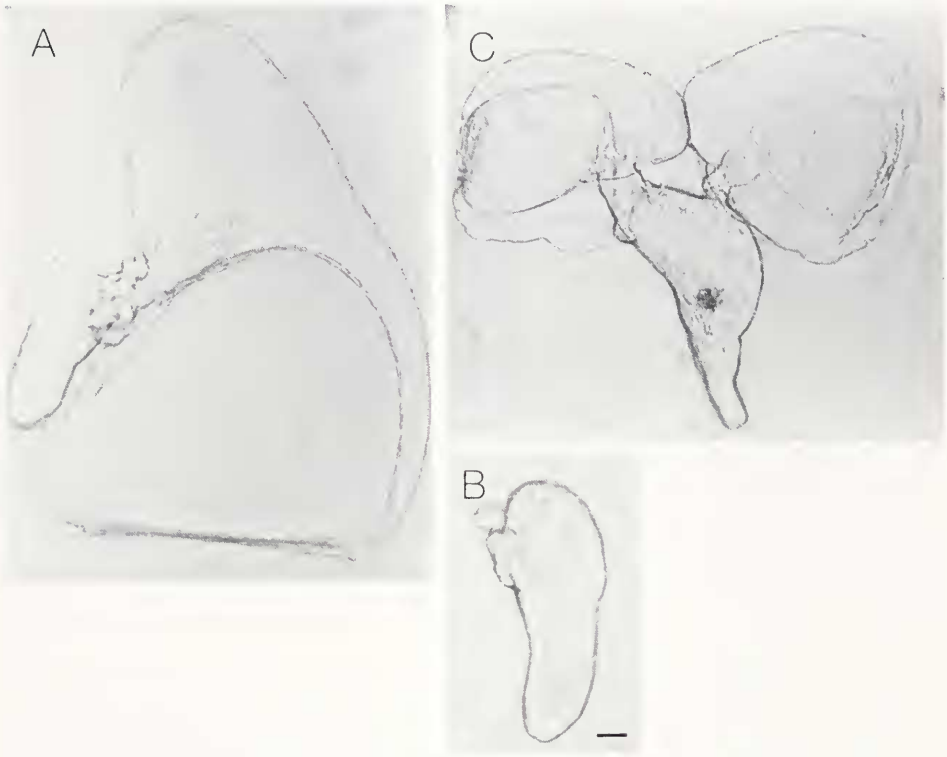

FIGURE 16. *Muggiaea* larvae from operated embryos. A) Six day old ventral isolate from 22 hour embryo. B) Six day old dorsal isolate from 22 hour embryo. Note the tentacle rudiment. The dorsal half is from the same embryo as (A). C) Five day old larva with two nectophores. All photographs are at the same magnification. The bar indicates 50 μm.

siphonophore egg in development. Eggs were centrifuged to produce ectoplasmic and endoplasmic fragments. These experiments were only done on *Nanomia*. The endoplasmic fragments that were produced moved to the air water interface either during centrifugation or shortly after centrifugation and were destroyed. Figure 17a shows

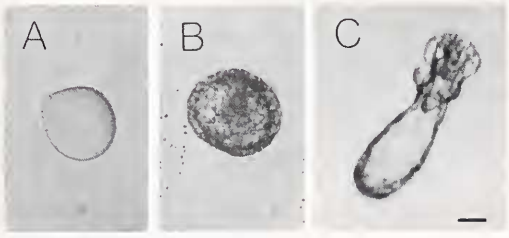

FIGURE 17. The development of ectoplasmic fragments and older *Nanomia* embryos that have lost their endoplasm. A) Ectoplasmic fragment from centrifuged egg. B) Five day ciliate sphere from ectoplasmic fragment. C) Seven day larva from embryo which lost its endoplasm at 16 hours of development. The embryo has a pneumatophore rudiment, vacuolated anterior cells, a tentacle rudiment and a mouth. It is much smaller than a normal larva. Compare this figure with 61. All photographs are at the same magnification. The bar indicates 50 μm.

an ectoplasmic fragment. The average diameter of these fragments was 131 μm (range 107–142 μm, sample size 12). An ectoplasmic fragment contains about 10% of the egg volume. Only about a fourth of the eggs that were centrifuged produced ectoplasmic fragments. Most (88%) of the ectoplasmic fragments cleaved. The early cleavages were normal. Cilia developed by 15 hours, however, the embryos did not elongate and there was no indication of swimming polarity. Twenty embryos were raised for five days. Ten of these cases were sectioned. There was no indication of organogenesis (Fig. 17b). This experiment suggests that the endodermal plasm is necessary for normal development.

This conclusion is supported by observations on post gastrula *Nanomia* embryos that get caught on the air-water interface. When this happens most or all of the endodermal cells are lost and one is left with an ectodermal hull. Unfortunately this procedure for removing the endoderm of the embryo is not exactly well controlled. When an embryo is de-endodermized between 6 and 12 hours of development (5 cases) the ectoderm that remains forms a ciliated ball and no organogenesis occurs. If even a small amount of endoderm remains the embryo will show swimming polarity and a mouth and/or a few large vacuolated cells will form (12 cases). If an embryo is de-endodermized after it has begun to elongate it will differentiate most structures even though the larva will be very small (8 cases) (Fig. 17c). This suggests that the yolky endoderm is only necessary for the early stages of development.

Discussion

The generality of the findings

Trachylina. There are a number of descriptive studies on early development of other species in the order Trachylina. Most of this literature dates from the last century; a great deal of it is summarized in Metschnikoff's (1886) monograph on the embryology of medusae. This monograph describes the egg and/or early developmental stages of seven species in the order Trachylina; it also provides comparative data on a number of species in the order Hydroida. All of the species in the order Trachylina appear to have eggs with large endoplasmic granules; these granules are much smaller in the eggs and early developmental stages of species in the order Hydroida. Within the order Trachylina there appears to be some variation in the size of these granules and their packing in different species.

In all of the species in the order Trachylina there appears to be an early establishment of ectodermal and endodermal cell layers. In *Aglantha* this process begins at the eight cell stage: I suspect that this may also occur at this stage in two of the species Metschnikoff studied, *Aglaura* and *Polyxenia.* Prior to the work on *Aglantha* described here, gastrulation was considered to be the time when ectodermal and endodermal cell layers formed. Gastrulation can occur in several ways in cnidarians; several schemes describe the ways in which this process can occur (Tardent, 1978). Different species in the Trachylina have been placed in different slots in these schemes. However in every case gastrulation involves a delamination in which a cell division takes place in such a way that an inner larger cell inherits primarily the granular endoplasm and a smaller outer daughter cell inherits primarily the cortical cytoplasm. This type of gastrulation is not too different from the formation of an endoplasm-poor micromere at the eight cell stage. In every case these gastrulation events occur at an early stage of development before a great deal of cell division has taken place. The kinds of cnidarian gastrulation that are associated with later cleavage stages, such as ingression and secondary delamination, do not occur in these embryos.

The process of embryogenesis in *Aglantha* is very similar to the process of em-

bryogenesis in *Aglaura* which Metschnikoff (1886) has studied. Metschnikoff considered the possibility that epiboly might occur in *Aglaura* but rejected it because he had no evidence that smaller blastomeres were moving over the larger blastomeres; however, I have seen epiboly occur in *Aglantha.* The fact that two cell stage blastomere isolates sometimes have an ectodermal cap at their aboral end supports this view—these cases would be generated when epiboly does not occur.

At this point no experiments have been done on the embryos of other species in the order Trachylina that elucidate the issues considered here. Both Maas (1908) and Zoja (1895) separated and reared blastomeres from early cleavage stage embryos of the narcomedusae *Liriope* and *Geryonia.* Single blastomeres isolated from two and four cell stage embryos form medusae or medusa larvae.

Siphonophora. The early development of only a few species of siphonophores has been studied. Carré's papers (1967, 1969) contain the best histological descriptions of early development. All species of siphonophores appear to have relatively large eggs. In every case where the egg has been examined, it contains relatively large endoplasmic granules and there is a sharp boundary between the endoplasm and the cortical layer of the egg. In every case where gastrulation has been described, it appears to take place early in development by delamination.

The only experimental work on early embryogenesis in siphonophores has been done by Carré (1969) on *Nanomia bijuga.* This work addresses the issue of whether regulation can occur along the oral-aboral axis of the embryo and the effect of developmental age on regulatory ability. The results she obtained contradict the results presented here. Because her work is only briefly described, many crucial details that would aid in interpreting the experiments are not given. One set of experiments involved the isolation of blastomeres at the 2, 4, 8, and 16 cell stages. In a crucial experiment an eight cell stage embryo was separated into eight blastomeres; seven of these isolates formed a pneumatophore bud and a tentacle. Another set of experiments divided gastrulae, young planulae, and planulae with a pneumatophore bud into two halves. In the experiment on the planulae with a pneumatophore bud, and presumably, in the young planulae, the cut created oral and aboral halves, but when gastrulae were cut into halves the cut was not oriented because marked embryos would have to be used. Carré reports that when gastrulae or young planulae were cut in half regulation occurred in all cases. The only developmental stage where regulation did not occur was the planula with the pneumatophore bud. At this stage the aboral half developed into a small siphonula without a gastrozoid and the oral half formed a gastrozoid but did not differentiate a pneumatophore. Carré concluded that regulation is total in young planulae and disappears when organogenesis begins.

It is hard to believe that two species belonging to the same genus should behave in such different ways. At present on the basis of my experiments on *Nanomia cara* and *Muggiaea* I would argue that there is probably an early specification of different regions along the oral-aboral axis of all siphonophore embryos. However Carré's report suggests that the situation may be more complex. It is conceivable that some species in this Order may show an early specification of different regions along the oral-aboral axis of the embryo and that these same species may differ in their ability to regulate. In other species the ability of different regions along the oral-aboral axis to regulate may be so extensive that it may be difficult to define when a particular region along this axis is specified.

The comparative embryology of the Hydroida and Ctenophora

Hydrozoans with both direct and indirect development and ctenophores share a number of developmental traits. Both of these groups have a centrolecithal egg with

a central yolky endoplasmic region that is surrounded by a peripheral layer of cortical cytoplasm. In both groups cleavage is unipolar. The oral-aboral axes of the embryos are established at the time of first cleavage under conditions where the oral pole of the axis corresponds to the site of first cleavage initiation (Freeman, 1977, 1980). In the order Hydroida this region corresponds to the posterior end of the planula which becomes the mouth of the polyp after metamorphosis.

When the basic features of development in the order Hydroida are compared with a similar set of features in the Ctenophora, several major differences between these two groups that involve the structure of the egg, the process of embryogenesis and the mechanisms that underlie this process become apparent. Each of these differences will now be examined.

Egg organization. While the Hydroida and Ctenophora have centrolecithal eggs, these two groups differ in the way this organization is expressed. The endoplasmic granules of ctenophore eggs are larger and more closely packed than those of Hydroida eggs, as a consequence the transition between the ectoplasmic and endoplasmic regions is much sharper in ctenophore eggs (see Fig. 30 in Freeman and Reynolds, 1973 for a section through a typical ctenophore egg and Fig. 1 in Freeman and Miller, 1982, for sections through Hydroida eggs.). In the ctenophore egg both of these cytoplasmic layers behave to a large extent like immiscible fluids (Spek, 1926). This kind of cytoplasmic behavior appears to be absent or much less pronounced in the Hydroida.

The *Aglantha* egg is similar to a ctenophore egg in that it has large endoplasmic granules; however these granules are not closely packed. Nevertheless the ectoplasmic region of the *Aglantha* egg appears to be more distinct than it is in Hydroida eggs. Both siphonophore eggs have large closely packed endoplasmic granules (see Fig. 1 in Carré and Sardet, 1981, for sections through the egg of a related species of *Muggiaea*) and a distinct ectoplasmic region. These eggs closely resemble ctenophore eggs.

Cleavage pattern. In the Hydroida it is difficult to talk about cleavage patterns during early embryogenesis. After the first cleavage there is generally not a set orientation for subsequent cleavage furrows, even though certain cleavage planes are more probable than others. There is no evidence that ectoplasm and endoplasm are differentially distributed to different blastomeres during early cleavage (Tardent, 1978). In ctenophores early cleavage occurs according to a stereotypic pattern. The first three cleavages take place along the oral-aboral axis of the embryo generating eight macromeres. Ctenophores are biradially symmetrical; there is a one-to-one relationship between the planes of the first cleavages and the sagittal and tentacular planes of these embryos. At the fourth cleavage each macromere gives off a micromere at the aboral pole of the embryo. During this division there is a differential distribution of cytoplasm so that the micromeres inherit very little endoplasm. During the next few divisions additional yolk-free micromeres are given off at the aboral pole of the embryo. These micromeres will become the ectodermal covering of the embryo; gastrulation occurs by epiboly (Reverberi, 1971).

In *Aglantha* the initial cleavage divisions also generate a stereotypic pattern. This embryo closely resembles the ctenophore embryo in that micromeres which are largely yolk-free are generated at the aboral end of the embryo. These micromeres will also form at least part of the ectodermal covering of the embryo. Gastrulation takes place in the same way in both forms. The two siphonophores do not generate a stereotypic cleavage pattern, in this sense they are Hydroida-like. However, in *Muggiaea* the plane of the first cleavage corresponds to the plane of bilateral symmetry of the embryo. Thus there is a relationship between the plane of cleavage and a symmetry property of the embryo as there is in ctenophores. Both siphonophore embryos undergo differential divisions at early stages of development that generate endoplasm and

ectoplasm free cells. These divisions which constitute gastrulation produce the external ectoplasm containing cells that form the ectoderm of the embryo; although this differential division does not occur in the same way it does in the ctenophore embryo, it has the same effect.

The establishment of embryonic regions with different developmental potentials. During the early cleavage stages of embryogenesis in ctenophores several cell divisions have been identified that give rise to daughter cells with different developmental potentials (Reverberi, 1971; Freeman and Reynolds, 1973). The first of these divisions occurs at the third cleavage. If a blastomere is isolated at the four cell stage it will continue to cleave and subsequently differentiate comb plate cilia cells and light producing cells. When the four cell stage blastomere divides, it produces E and M daughter cells. If the E blastomere is isolated it will subsequently differentiate comb plate cilia cells, but not light producing cells, while the isolated M macromere will differentiate light producing cells but not comb plate cilia cells. In these embryos cleavage does not passively divide up special cytoplasmic regions of the egg that have been in place for some time. The factors that specify these two cell types are gradually localized in the future E and M macromere forming regions of the embryo during the two cleavages which precede this division (Freeman, 1976). These embryos behave like a mosaic of parts which have been largely specified during early cleavage stages.

In the Hydroida that gastrulate by unipolar ingression it is possible to map the position of the presumptive ectodermal and endodermal cells prior to gastrulation. The ectodermal cells are found at the presumptive anterior end while the endodermal cells are found at the presumptive posterior end of the embryo. At any time prior to gastrulation it is possible to isolate each of these presumptive regions and both kinds of isolates will form a normal planula larva with both ectodermal and endodermal cell layers (Freeman, 1981). When an isolated region regulates to form a normal planula it always conserves its polarity properties (Teissier, 1931). During gastrulation the presumptive ectodermal cells lose their capacity to form endodermal cells; this is the first point during development where there is a restriction of developmental potential (Freeman, 1981). Following gastrulation the embryo differentiates into a planula larva. If a post gastrula embryo or planula is cut up into regions with different presumptive fates, each part will regulate to form a normal planula, as long as both ectodermal and endodermal cell layers are present (Müller *et al.*, 1977; Freeman, 1981, however, see Lesh-Laurie, 1976). These embryos behave like developmental fields (Wolpert, 1969). The way a given cell differentiates in these embryos ultimately depends upon its position with respect to its neighbors.

In *Aglantha* and the two siphonophores there is an early specification of different embryonic regions. In *Aglantha* the micromeres that are produced at the eight cell stage differentiate only ectoderm while the macromeres differentiate both ectoderm and endoderm. In ctenophores the micromeres and macromeres produced at the 16 cell stage differentiate in the same way. This is quite different from the Hydroida where ectoderm and endoderm are not specified until gastrulation. Gastrulation in the hydroida is not an early event as it is in *Aglantha,* but a relatively late event, at least in those forms which gastrulate by ingression. After gastrulation the aboral half of the *Aglantha* embryo cannot regulate to form a mouth and tentacles; it behaves differently from the aboral half of the Hydroida embryo which can regulate. Unfortunately this experiment has not been done on ctenophore embryos.

In *Nanomia* there is a specification of different regions along the oral-aboral axis of the embryo by the eight cell stage; this specification occurs before ectodermal cells have formed as it does in the ctenophore embryo. In *Muggiaea* the situation is more complicated, while the aboral region of the embryo is specified by the eight cell stage,

the oral part of the embryo is capable of regulation until just before organogenesis begins; the same is true of the presumptive dorsal half of the embryo. In this embryo the timing of determinative events appears to be a mix which has some of the elements of the ctenophore situation and some of the elements of the Hydroida situation.

The role of ectoplasm and endoplasm in cell specification. In both the Ctenophora and the Hydroida, experiments have been done to create egg fragments that lack endoplasm (see Beckwith, 1914; Freeman and Miller, 1982, for the Hydroida, and LaSpina, 1963; Freeman and Reynolds, 1973 for the Ctenophora). This experiment is done by centrifuging fertilized uncleaved eggs to stratify the egg contents and then increasing the centrifugal force or cutting the egg to give a nucleated ectoplasmic fragment. When this experiment is done on ctenophores the initial cleavages are normal. However there is not a normal segregation of developmental potential, both the E and M macromeres differentiate comb plate cilia. These embryos fail to differentiate certain cell types such as light producing cells and they develop into a poorly organized ectodermal mass (see Fig. 35 in Freeman and Reynolds, 1973 for a cross section through one of these "embryos"). In the Hydroida ectoplasmic fragments form normal planulae. This comparison indicates that endoplasm is necessary for normal embryogenesis in the Ctenophora, but not in the Hydroida.

Ectoplasmic fragments of both *Aglantha* and *Nanomia* differentiate ectodermal masses that are similar to the ectodermal mass produced under similar conditions by the ectoplasmic fragments of Ctenophore eggs. The behavior of the ectoplasmic fragments reflects the marked distinction between the ectoplasm and the endoplasm in the eggs and embryos and the inheritance of the ectoplasm by the ectodermal cells in these three groups of animals. The lack of morphogenesis in these ectodermal masses probably reflects the lack of endoderm. Hydroida embryos which lack endoderm are capable of undergoing metamorphosis but cannot form a polyp (Freeman, 1981).

This comparison of development in the Ctenophora, the Hydroida, the Trachylina, and the Siphonophora shows that the Trachylina and the Siphonophora each have an egg organization, a mode of early development, and a set of mechanisms for specifying embryonic regions that is very similar to those found in Ctenophores.

The bases for developmental parallelism

The Cnidaria and the Ctenophora are thought to be closely related (Hyman, 1940). It is possible that the development parallelism between the Trachylina, the Siphonophora, and the Ctenophores could be explained on the basis of common descent. At present there is no agreement about how the classes and orders in the phylum Cnidaria are related. It is not even clear what the most primitive members of the phylum looked like. Some students of this group have argued that the first Cnidarians were polyps (Werner, 1973) while others have argued that the first Cnidarians were medusae (Brooks, 1886, Rees, 1966). It is also not clear how the phylum Ctenophora is related to the Cnidaria. However, a number of speculative phylogenies have been developed that have the status of educated guesses. Hyman (1940) has argued that the Trachylina and the Ctenophora are closely related. No one has suggested the Siphonophora are closely related to either the Ctenophora or Trachylina. The speculations concerning the origin of the Siphonophora derive this order from the Hydroida (Totton, 1965).

This parallelism may also reflect the fact that these embryos develop directly. During embryogenesis a set of structures are going to develop which are more elaborate than those of a planula larva. This reflects the fact that these animals have to function in a pelagic environment. It will take a certain amount of time to generate these

structures. Because the egg is a closed system, only so much time is available for building these structures before the embryo's nutrient reserves are depleted. These two considerations could place a premium on the way time is allocated during embryogenesis.

Before a structure develops a decision has to be made about its placement. An embryonic field is one mechanism for specifying structure placement and is used by the Hydroida. This mechanism relies on physiological machinery which assigns each cell an address with respect to its neighbors. In order for this mechanism to function, its physiological machinery has to be created and it must function for a period of time. This means that this could be a relatively costly mechanism in terms of time utilization. However, if the differentiation of a structure depends on the inheritance of localized cytoplasmic regions, as it appears to be in the direct developers, the time needed to decide where a given structure will be placed is reduced substantially.

The process of embryogenesis in Cnidarians also depends upon interactions between ectodermal and endodermal cell layers. This means that these cell layers have to exist before structure formation can begin. When the specification of these cell layers depends upon the position of a given cell with respect to its neighbors, as it does in the Hydroida with indirect development, this process is going to take much longer than it will in direct developing embryos where the parcelling out of ectoplasm and endoplasm at cleavage accomplishes the same end.

Embryonic field mechanisms and cytoplasmic localization mechanisms are frequently regarded as separate and distinct ways of specifying the developmental potential of different parts of embryos. The experiments described here suggest that during the course of evolutionary divirsification within a group of animals, a transition from one mechanism to the other can occur relatively easily (see Freeman, 1982, for a general discussion of this mode of evolutionary change).

The developmental similarities that the Trachylina, the Siphonophora, and the Ctenophora share is impressive. If one assumes that all three groups evolved independently from a Hydroida like stock, one would have to argue that while there are no constraints which prevent the transition from a field to a cytoplasmic localization mechanism of embryonic determination, the way one undergoes the transition is highly constrained. For example, in all three groups the axial relationships are similar, and when cleavage is related to symmetry, the same relationship holds in different groups. This kind of constraint provides a basis for explaining the developmental similarities within these groups.

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